



Interaction of central kisspeptin with melanocortin, GABAergic, corticotrophin, and NPY systems on food intake in chickens

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ABSTRACT

Kisspeptin is a key component of reproduction that can directly affect food intake in mammals. There is evidence suggesting that melanocortin, GABA, corticotrophin, and neuropeptide Y (NPY), have a mediatory role in reward; however, how these substances interact with kisspeptin-induced by food intake in birds, remains to be identified. Accordingly, in this study, a total of 10 experiments were carried out to investigate the interplay between kisspeptin and these systems for the control of food intake in neonatal layer-type chickens. In the first experiment, chickens were intracerebroventricular (ICV) injected with saline and Metastatin (Kisspeptin, 0.25, 50, and 1 nmol). In the second experiment, saline, Metastatin (1 nmol), BIBP-3226 (NPY1 receptor antagonist, 1.25 nmol), and co-injection of Metastatin + BIBP-3226 were injected. Experiments 3-10 were similar to experiment 1, except that chickens received BIIE 0246 (NPY2 receptor antagonist, 1.25 nmol), CGP71683A (NPY5 receptor antagonist, 50 µg), Picrotoxin (GABAA receptor antagonist, 1.25 nmol), CGP54626 (GABAB receptor antagonist, 21 µg), astressin-B (CRF1 / CRF2 receptor antagonist, 30 µg), Astressin2-B (CRF2 receptor antagonist, 30 µg), SHU9119 (MC3 / MC4 receptor antagonist, 0.5 nmol), and MCL0020 (MC3 / MC4 receptor antagonist, 0.5 nmol) instead of BIBP-3226. Food intake was subsequently assessed until 120 min after the injection. Based on the findings, Metastatin (0.25, 50, and 1 nmol) significantly increased food intake in a dose-dependent manner ($p < 0.05$). However, BIBP-3226 and Picrotoxin inhibited Metastatin-induced hyperphagia in neonatal chickens ($p < 0.05$); Whereas, whereas BIIE 0246, CGP71683A, CGP54626, astressin-B, astressin2-B, SHU9119, and MCL0020 had no effect ($p > 0.05$). These results showed that the effect of kisspeptin on food intake might be mediated by NPY1 and GABAA receptors in layer-type chickens.

Keywords

Kisspeptin; GABA, Melatonin; NPY; Corticotropin; Dietary intake; Layer-type chicken

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Abbreviations

NPY: Neuropeptide Y
ICV: Intracerebroventricular
ARC: Arcuate nucleus: ARC

NTs: Neurotransmitters
Paraventricular nuclei: PVN
Agouti-related protein: AgRP

Introduction

Appetite control in animals is a complicated process involving both the central nervous system (CNS) and the peripheral nervous system (PNS), modulated by various factors. The hypothalamic regions, particularly the amygdala, nucleus tract, and arcuate nucleus (ARC), can control the appetite via various neurotransmitters (NTs) in CNS [1]. RF amides comprise some peptides with a similar amino acid sequence at the C-terminus, which can play a role in feeding behavior [2]. Also, it has been documented that RF amides regulate food intake in chickens [3], rodents [4], and humans [5]. Several metabolic activities are provided by Metastin (kisspeptin-10), which is expressed abundantly in the placenta, testis, spine, pancreas, pituitary, and hypothalamic regions [6]. Metastin is expressed in food intake regulated by regulating nuclei, such as ARC and paraventricular nuclei (PVN), and can play a regulatory important role in food intake and energy homeostasis [7]. In this regard, Khan et al (2009) reported that ICV administration of Metastin increases food intake in chickens [6].

The ARC functions as a nucleus to control appetite and can facilitate the relationship between appetite-sensing cells in mammals and birds. The appetite is regulated by NPY/agouti-related protein (AgRP), proopiomelanocortin (POMC), and cocaine/amphetamine-regulated transcript (CART) [8]. NPY has six receptors, and NPY1 and NPY5 receptors are responsible for feed intake regulation. However, NPY2 is an autoreceptor that can influence appetite in food-deprived animals. Previously, Yousefvand et al. (2019 and 2020) reported that the ICV administration of the NPY1 and NPY5 receptors antagonists, including B5063 and SML0891, decreased food intake in a dose-dependent manner, while SF22 (an antagonist for NPY2 receptor) increased food intake in broiler chickens [9, 10]. Numerous physiological processes, including cleanliness, temperature homeostasis, training, and energy balancing, are mediated by the melanocortin pathway in the CNS [11]. So far, five melanocortin receptors have been discovered, from MC1R to MC5R; however, the fundamental role of food consumption only includes MC3R and MC4R isoforms [12]. There are many areas of the hypothal-

amus involved in energy homeostasis and food consumption, such as the ARC, ventromedial hypothalamus (VMH), and PVN, which include the MC3R and MC4R [11, 12, 13].

In their study Previously, Bonaventura et al. (2020) showed that ICV administration of agonists and antagonists of the MC3/MC4 receptors reduced and enhanced, food intake, respectively [12]. According to a study conducted by Ahmadi et al. (2019), MC3 and MC4 receptors have a regulatory effect on dietary consumption. The primary process during distress sensitivity is mainly handled by a peptide comprised of 41 amino acids called corticotrophin-releasing factor (CRF). Corticotrophin receptors, especially CRF1 and CRF2, are capable of strong anorectic and thermogenic effects [14]. The CRF1/CRF2 antagonist astressin-B or the CRF2 antagonist astressin2-B abolished icv nesfatin-1's anorexigenic action, whereas an astressin2-B analog, devoid of CRF-receptor binding affinity, did not ICV administrations of adjusted of food in rats [15]. Activation of brain CRF signaling pathways by CRF acting on CRF1 and CRF2 receptors inhibits food intake regulated [16]. Also, Heidarzadeh et al. (2017) showed that astressin-B inhibits hypophagia caused by nesfatin-1 in 3h- food-deprived (FD3) broiler chickens.

In their study Fu et al. (2010) reported that Metastin (0.3, 1, and 3 µg/mouse) dose-dependently inhibited the food intake to overnight fasting in mice, and there was an interconnection between Metastin and NPY, and POMC, as Metastin activates POMC, whereas NPY neurons are inhibited [17]. Furthermore, orexigenic melanin-concentrating hormone (MCH) activates neurons inhibited by Kisspeptin [18]. There is a scarcity of data on the effects of Metastin on food intake in chickens because most knowledge on this peptide is sourced from mammalian species.

There is no information about the interplay of metastin with melanocortin, GABAergic, corticotrophin, and NPY systems in the CNS of birds. A novel model to investigate the impacts of a peptide influencing both appetite and adiposity is the use of a line of chickens that vary in these characteristics. The low-weight strain was selected for lower food consumption and raised for egg production, whereas the high-weight strains have higher food consumption which they are famous for meat production [19, 20]. In the poultry industry, studies on layer-type chickens aim to boost the productivity of hens by decreasing food intake and malnutrition. It is critical to understand the potential interactions of kisspeptin with other neurotransmitters in poultry based on comparative physiology. Therefore, this study aimed to investigate the mediatory effects of the central melanocortin, GABAergic, corticotrophin, and NPY systems on kis-

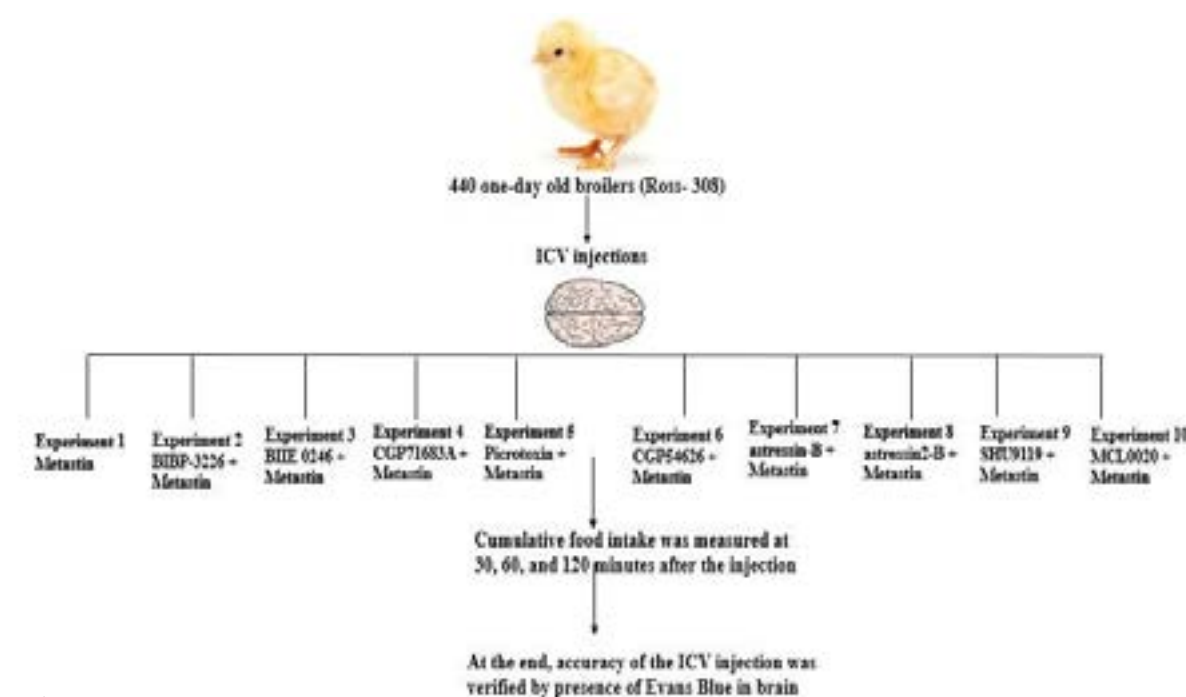


Figure 1.
Flow chart of the experimental procedure.

speptin induced- by food intake in neonatal layer-type chickens.

Results

In experiment 1, chicks treated with Metastin (Kisspeptin 0.25, 50, and 1 nmol) increased food intake at 30, 60, and 120 min post-injection ($p < 0.05$) (Figures 1 and 2).

In experiment 2, chicks treated with Metastin (1

nmol) increased dietary consumption at 30, 60, and 120 min post-injection ($p < 0.05$). No significant difference was observed between BIBP-3226 (1.25 nmol) and control groups in dietary consumption of chickens ($p > 0.05$). Co-injection of the Metastin + BIBP-3226 inhibited Metastin-induced hyperphagia ($p < 0.05$) (Figure 3).

In experiment 3, Metastin (1 nmol) increased dietary intake at 30, 60, and 120 min post-injection ($p < 0.05$).

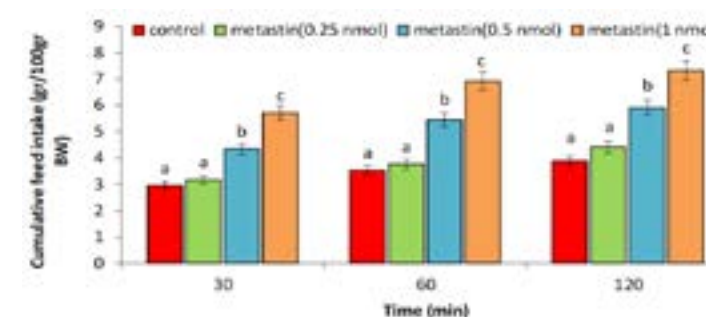


Figure 2.
Effect of ICV injection of metastin (0.25, 0.5, and 1 nmol) on cumulative food intake in neonatal chicken (n = 44). metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a, b, and c) indicate significant differences between treatments ($p < 0.05$).

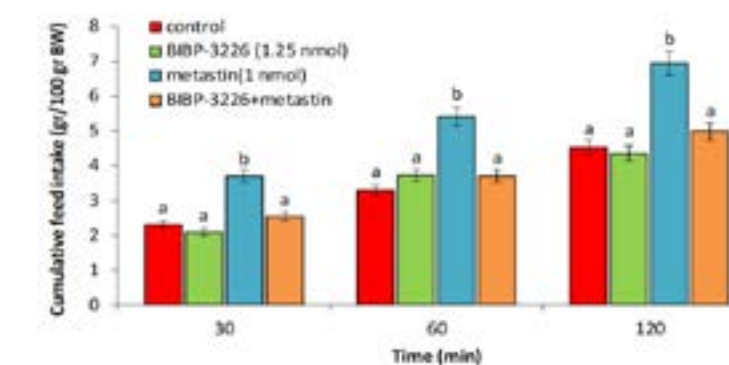


Figure 3.
Effect of ICV injection of BIBP-3226 (1.25 nmol), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). BIBP-3226: NPY1 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

Abbreviations-Cont'd

Proopiomelanocortin: POMC
Ventromedial hypothalamus: VMH
Food-deprived: FD3
Brain-Derived Neurotrophic Factor: BDNF
Gonadotropin-inhibiting hormone: GnIH
Analysis of variance: ANOVA
BDNF: Brain-Derived Neurotrophic Factor
GnIH: Gonadotropin-inhibiting hormone

0.05). Also, no difference was found between the BIIE 0246 and control groups ($p > 0.05$). Metastin + BIIE 0246 co-administration could not alter the hyperphagic effect caused by Metastin ($p > 0.05$) (Figure 4).

In experiment 4, chickens treated with Metastin (1 nmol) increased dietary intake at 120 min after the administration ($p < 0.05$). However, CGP71683A (50 μ g) was not correlated with a significant change in food intake at 120 min after the administration ($p > 0.05$). In addition, Metastin + CGP71683A co-administration did not affect Metastin-induced hyperphagia ($p > 0.05$) (Figure 5).

In experiment 5, ICV administration of the Metas-

tin (1 nmol) enhanced food intake at 30, 60, and 120 min post-injection ($p < 0.05$). At 120 min after the administration of Picrotoxin (1.25 nmol), no significant impact on food intake was reported ($p > 0.05$). However, Metastin plus Picrotoxin co-administration attenuated Metastin-induced hyperphagia ($p < 0.05$) (Figure 6).

In experiment 6, co-administration of Metastin plus CGP54626 (21 ng) was not associated with hyperphagic effects caused by Metastin ($p > 0.05$) (Figure 7).

In experiment 7, Metastin (1 nmol) increased food intake in chickens ($p < 0.05$). Also, no significant effect on food intake was observed at 120

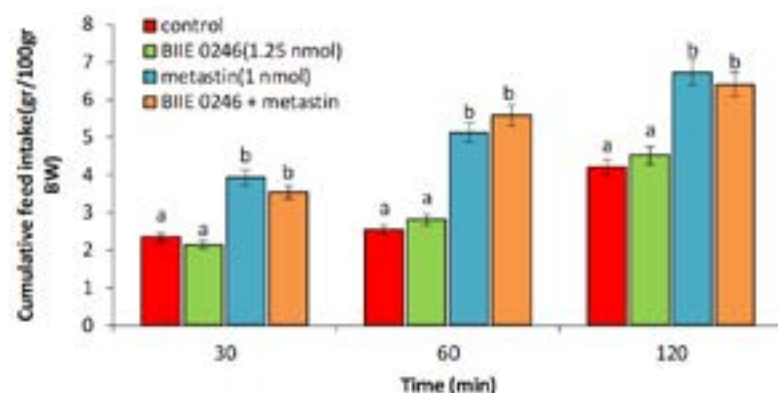


Figure 4. Effect of ICV injection of BIIE 0246 (1.25 nmol), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). BIIE 0246: NPY2 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean \pm SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

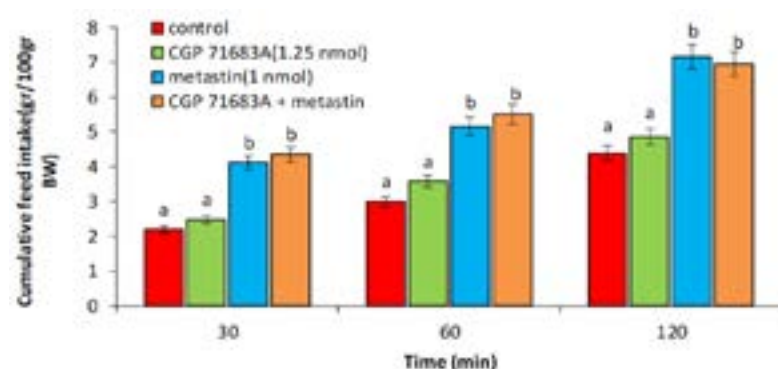


Figure 5. Effect of ICV injection of CGP71683A (1.25 nmol), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). CGP71683A: NPY5 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean \pm SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

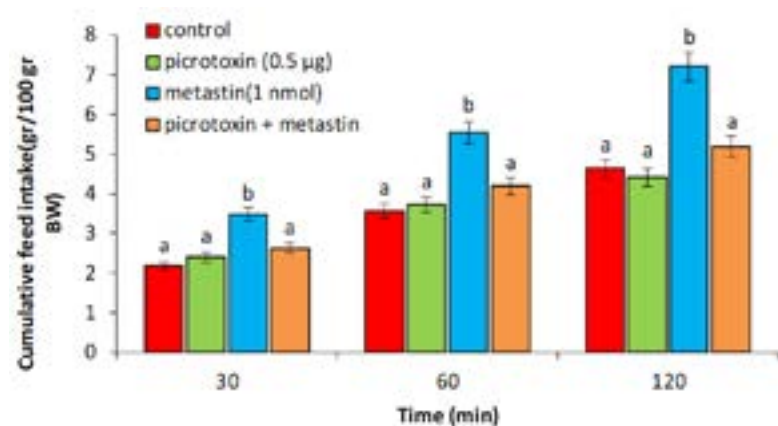


Figure 6. Effect of ICV injection of picrotoxin (0.5 μ g), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). picrotoxin: GABAA receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean \pm SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

min following the administration of Astressin-B (30 μ g) ($p > 0.05$). No significant impact on food intake was identified at 120 min after the co-administration of Metastin + astressin-B ($p > 0.05$) (Figure 8).

In experiment 8, the injection of Metastin (1 nmol) increased dietary intake ($p < 0.05$). At 120 min after the administration of Astressin-2B (30 μ g), no significant impact was observed on dietary consumption ($p > 0.05$). At 120 min following co-administration of Metastin + astressin2-B, no significant effect on food consumption was observed in comparison with Metastin group ($p > 0.05$) (Figure 9).

In experiment 9, ICV administration of the Metastin (1 nmol) increased food intake at 120 min after the injection ($p < 0.05$). However, at 120 min

following the administration of SHU9119 (0.5 nmol), no significant impact on food intake was reported ($p > 0.05$). In addition, co-administration of Metastin + SHU9119 could not alter hyperphagic effects caused by Metastin ($p > 0.05$) (Figure 10).

In experiment 10, chicks treated with Metastin (1 nmol) enhanced food intake at 120 min after the injection ($p < 0.05$). At 120 min after administration of MCL0020 (0.5 nmol), no significant change was observed in food intake ($p > 0.05$). Also, co-administration of Metastin + MCL0020 could not alter the hyperphagic effect of Metastin in chickens ($p > 0.05$) (Figure 11).

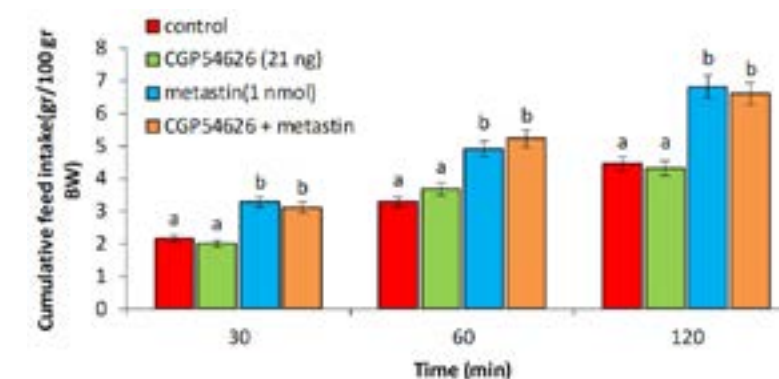


Figure 7. Effect of ICV injection of CGP54626 (21 ng), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). CGP54626: GABAB receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean \pm SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

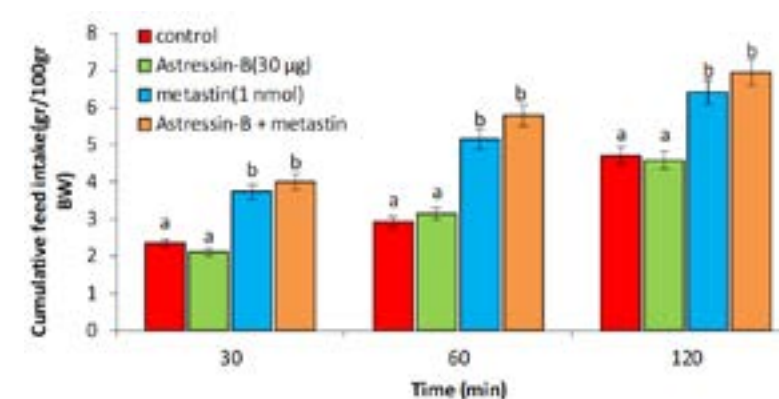


Figure 8. Effect of ICV injection of astressin-B (30 μ g), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). astressin-B: CRF1/CRF2 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean \pm SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

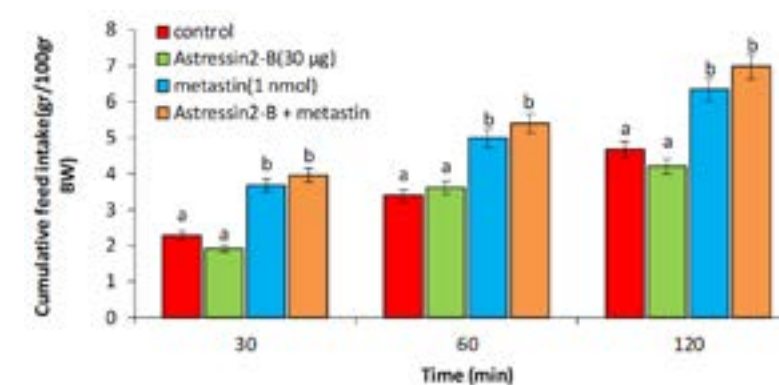


Figure 9. Effect of ICV injection of astressin2-B (30 μ g), metastin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). Astressin2-B: CRF2 receptor antagonist, metastin: kisspeptin-10. Data are expressed as mean \pm SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

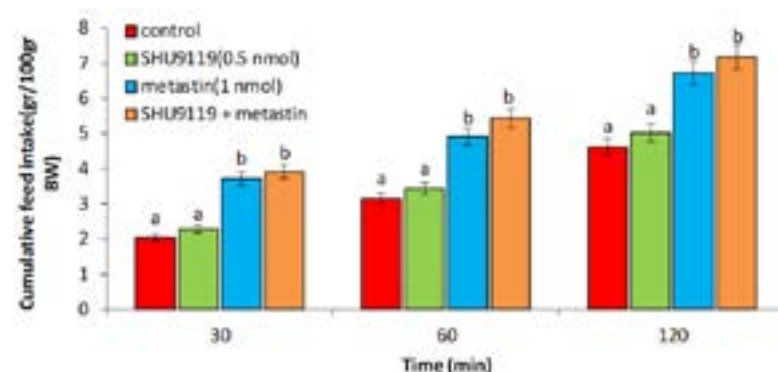


Figure 10. Effect of ICV injection of SHU9119 (0.5 nmol), metastatin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n = 44). SHU9119: MC3/MC4 receptor antagonist, metastatin: kisspeptin-10. Data are expressed as mean \pm SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

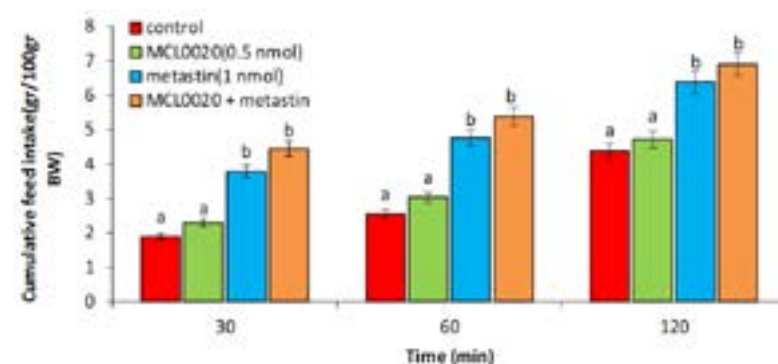


Figure 11. Effect of ICV injection of MCL0020 (0.5 nmol), metastatin (1 nmol), and their combination on cumulative food intake in neonatal chicken (n=44). MCL0020: MC4 receptor antagonist, metastatin: kisspeptin-10. Data are expressed as mean \pm SEM. Different letters (a and b) indicate significant differences between treatments ($p < 0.05$).

Discussion

In the present study, we show for the first time that Metastin injected into the lateral brain ventricle at doses (0.25, 50, and 1 nmol) increased food consumption in a dose-dependent manner in neonatal chickens. ICV administration of the Metastin increased dietary consumption in chickens [6]. Stengel et al. (2011) reported that Kisspeptin-10 (0.3, 1, and 3 μ g/mouse) dose-dependently inhibited the feeding response to an overnight fast by 50%, 95%, and 90% respectively during the 2–3 h period post injection. The 1 μ g/mouse dose reduced the 4-h cumulative food intake by 28% while intraperitoneal injection (10 μ g/mouse) did not [7]. Controversial reports exist regarding the role of Kisspeptin as an orexigenic or an anorexigenic factor. For example, One cell research revealed that kisspeptin increases the expression of neuropeptide Y (NPY) which is known to stimulate food intake [27, 29]. In contrast, other authors report that this peptide has an excitatory effect on anorexigenic POMC neurons [17]. Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism [17]. Stengel et al. (2011); showed that the centrally injected Kisspeptin reduced food intake by increasing meal intervals in mice [7]. However, central injection of kisspeptin is not able to significantly alter the pattern of food intake, either in rats fed ad libitum or subjected to the previous 12 h of fasting. In good

agreement, intracerebral administration of kisspeptin, at a dose effective to maximally elicit LH secretion, failed to change hypothalamic expression levels of NPY, Agouti-related peptide, proopiomelanocortin, and cocaine- and amphetamine-regulated transcript mRNAs (unpublished data) [28]. Perhaps one of the reasons for these discrepancies is the differences between animal species and their physiological differences in regulating centers of food intake.

Co-administration of the Metastin plus NPY1 receptor antagonist inhibited Metastin-induced hyperphagia. Metastin and the NPY2 and NPY5 receptors were not shown to be interconnected during the control of dietary consumption in layer-type birds. Kisspeptin-10 increases the NPY gene expression while inhibiting the Brain-Derived Neurotrophic Factor (BDNF). It is well established that NPY and Kisspeptin neurons could play a critical role in the neural system regulating the pulsatory secretion of GnRH. To understand how NPY regulates Kisspeptin neurons, it has been suggested to be a possible physiological modulator [29]. NPY and Kisspeptin neurons are close in the ARC, and KiSS-1 receptors are expressed in hypothalamic NPY neurons [30]. In addition, NPY null mice showed decreased KiSS-1 mRNA levels at the hypothalamus, whereas exposure to NPY increased the expression of KiSS-1 in hypothalamic N6 cells [31]. Kisspeptin inhibits orexigenic NPY neurons through an indirect mechanism based on enhancing GABA-mediated inhibitory synaptic tone. In

striking contrast, gonadotropin-inhibiting hormone (GnIH and RFRP-3) and NPY, also found in axons abutting POMC cells, inhibit POMC cells and attenuate the kisspeptin excitation by a mechanism based on opening potassium channels [17]. Also, Kisspeptin could directly regulate neuropeptide Y synthesis and secretion via the ERK1/2 and p38 mitogen-activated protein kinase signaling pathways in NPY-secreting hypothalamic neurons [30].

Moreover, central, but not peripheral, injection of kisspeptin-10 was found to decrease food intake in overnight fasted mice [32]. However, kisspeptin neurons are direct targets for regulation by leptin which could act at a post-transcriptional level [33], and it is well known that leptin plays a pivotal role in the hypothalamic regulation of feeding behavior, energy homeostasis, and reproduction [34,35]. Conversely, central injection of kisspeptin-10 was not found to affect feeding in rats [33,36]. Nevertheless, the kisspeptin gene resulted in upregulation in female rats fed on a cafeteria diet, further supporting a discrete role of kisspeptin in energy balance control [37]. Finally, food deprivation or other conditions of negative energy balance, including chronic calorie restriction, led to a significant reduction in hypothalamic KiSS-1 mRNA levels in rats [33,38]. Thus, the opposing findings between animal species might relate to different regulatory mechanisms for food intake among birds and mammals. Given the estimated 300 million years of evolutionary distance between mammals and avians, it is not surprising that significant differences have been found in the activities of several components involved in the regulation of energy homeostasis, such as ghrelin, leptin, and adiponectin [39].

As observed, co-administration of the Metastin plus GABAA receptors antagonist inhibited the hyperphagic effect of the Metastin. However, no interconnection was observed between Metastin and GABAB, CRF1 / CRF2, and MC3 / MC4 receptors on dietary intake regulation among layer-type chickens. It is reported that bicuculline (antagonist for GABAA receptors) could increase Kisspeptin release in the medial basal hypothalamus of prepubertal monkeys [40]. The rhythmic hypothalamic GABA emission variations mean that GABA neurotransmission blockage may be more efficacious in changing peptide secretion in prepubertal than mid-pubertal females [40]. Recently, Ibos et al. (2021) reported that Kisspeptin-8 induces anxiety-like behavior and hypolocomotion by activating the HPA axis and increasing GABA release in the nucleus accumbens in rats [41]. Kisspeptin-10 suppresses the gene expression of the CRF and increases arginine vasopressin and oxytocin in the PVN [41]. However, in the current study, no interconnection was observed between Metastin and CRF1 /

CRF2 receptors. As GABAA receptors were selectively activated, gonadotropin discharge was caused by Kisspeptin [42]. So, it is reported that GABA has a regulatory effect on gonadotropic responses to Kisspeptin in male rats [42]. The lateral hypothalamus is a key aspect in feeding and looking for a bonus. Further, the manipulation of GABA transmission in the lateral hypothalamus causes alterations in appetite since stimulation of GABAergic neurons promotes dietary intake, whereas the suppression of these neurons decreases dietary intake [43].

These results showed that the effect of Kisspeptin on food intake is mediated by NPY1 and GABAA receptors in layer-type chickens. The central eating pattern of rats has been the subject of many investigations. It is well established that central appetite control is different in mammals and birds [44]. Therefore, it is concluded that these activities in chickens are governed by the regulatory systems [45]. As observed, no prior study on food intake of the layer chickens was reported concerning Kisspeptin interactions with melanocortin, GABAergic, corticotrophin, and NPY systems. Thus, it was impossible to match our findings with other research. The results of the present study can be used as base information for further studies. Furthermore, it is required to perform merit studies to determine cellular and molecular mechanisms (s) involved in the interaction of Kisspeptin and NPY and GABAergic systems.

Materials & Methods

Animals

In this study, a regional incubator supplied a maximum of 440 one-day layer-type birds (Hy-line) (Morghak Co. Iran). For two days, chickens were stored as groups and randomly moved to isolated enclosures at a temperature of 30 ± 1 °C with $50 \pm 2\%$ humidity [21]. Chicks were fed a standard meal comprising 21% crude protein and 2850 kcal/kg metabolizable calories (Chineh Co. Iran). During the research, each chick received an ad libitum diet and fresh water. The birds were FD3; however, they had unrestricted availability to water approximately three hours before ICV administration. At 5 days old, chickens were randomly allocated into ten experiments with four groups (11 chickens per group).

Experimental drugs

Drugs included Metastin (Kisspeptin), BIBP-3226 (antagonist for NPY1 receptors), BIIE 0246 (an antagonist for NPY2 receptors), CGP71683A (antagonist for NPY5 receptors), Picrotoxin (antagonist for GABAA receptors), CGP54626 (antagonist for GABAB receptors), astressin-B (antagonist for CRF1/CRF2 receptors), Astressin2-B (antagonist for CRF2 receptors), SHU9119 (antagonist for MC3 / MC4 receptors), MCL0020 (antagonist for MC3/MC4 receptors) and Evans Blue were purchased from Sigma Co. (Sigma, USA). Medicines were initially dissolved in 100% dimethyl-sulfoxide (DMSO), followed by dilution with 0.85% saline comprised of Evans blue at 1/250 proportion. However, using DMSO in this proportion did not lead to cytotoxicity [22].

ICV administration procedures

To ensure that the average weight in treatment groups was as consistent as conceivable, the chickens were weighed before each administration and then divided into groups according to their body weight. The methods described by Davis et al. (1979) and Furuse et al. (1997) for the application of ICV injections with no anesthesia were performed using a microsyringe (Hamilton, Switzerland). Throughout the right ventricle, a microsyringe pierced 4 mm into the surface of the skull via the tip of the needle. When this technique was used on newborn chickens, it was discovered that no physiological stress was caused by injection [23]. Using an ICV administration with vehicle or medication preparation in a volume of 10 µl, all of the chickens were injected. Finally, the placement accuracy of the injection in the ventricle was verified by the presence of Evans Blue, followed by slicing of the frozen brain tissue [24].

Feeding experiments

In this study, a total of 10 experiments, each with four ICV intervention categories, were designed: 1-4 groups (n=44 in each). In experiment 1, birds received ICV administration of saline and Metastin (Kisspeptin 0.25, 50, and 1 nmol). In experiment 2, chickens were administered with saline, Metastin (1 nmol), BIBP-3226 (1.25 nmol), and co-administration of Metastin + BIBP-3226. In experiment 3, birds received the injection of saline, Metastin (1 nmol), BIIE 0246 (1.25 nmol), and co-administration of Metastin + BIIE 0246. In experiment 4, chickens received ICV injection of saline, Metastin (1 nmol), CGP71683A (50 µg), and co-administration of Metastin + CGP71683A. In experiment 5, chickens received the injection of saline, Metastin (1 nmol), Picrotoxin (1.25 nmol), and co-administration of Metastin + Picrotoxin. In experiment 6, ICV administration of the saline, Metastin (1 nmol), CGP54626 (21 µg), and co-administration of Metastin + CGP54626 was performed. In experiment 7, birds received saline, Metastin (1 nmol), astressin-B (30 micrograms), and co-administration of Metastin + astressin-B. In experiment 8, saline, Metastin (1 nmol), Astressin2-B, and Metastin plus Astressin2-B were administered. In experiment 9, chickens received the injection of saline, Metastin (1 nmol), SHU9119 (0.5 nmol), and co-administration of Metastin + SHU9119. In experiment 10, birds received the injection of saline, Metastin (1 nmol), MCL0020 (0.5 nmol), and co-administration of Metastin + MCL0020. The experimental procedure is shown in Figure 1. Dosage for injections was obtained from previous studies [6, 9, 12, 25, 26]. FD3 chickens were immediately transferred to their respective enclosures and given new water and meal (pre-weighed). Following the administration, the total food consumption (gr) was recorded at 30, 60, and 120 minutes. Consequently, food intake was measured as a percent of the body weight to reduce the impact of weight on the quantity of food consumed. Any chicken was just utilized once for statistical analysis in each treatment group. The data were expressed as mean ± SEM (standard error of the mean). The two-way repeated measures analysis of variance (ANOVA) using SPSS version 16.0 evaluated the total dietary consumption (as the bodyweight percent) (SPSS, Inc., Chicago, IL, USA). Averages were analyzed employing the Tukey-Kramer test for treatment showing a critical impact using ANOVA. Differences between ICV interventions were statistically significant at $p < 0.05$.

Agreement with ethical criteria

There is no conflict of interest between researchers. This article contains no research conducted by any of the authors with human participants investigations had been carried out in following the Guide for the Care and Use of Laboratory Animals and confirmed by the Committee for Institutional Animal Ethics.

Authors' Contributions

All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors have no conflicts of interest to declare.

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